Metabolites of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) in human urine after consumption of charbroiled or fried beef

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Abstract

Heterocyclic amines (HAs) are carcinogenic combustion products formed during the cooking of meat at moderate to high temperatures. PhIP is the most common HA formed in fried, grilled or broiled meat, and is a colon, breast, and prostate carcinogen in rodents. The major metabolites of PhIP detected in human urine are N^2-OH-PhIP-N^2-glucuronide, PhIP-N^2-glucuronide, N^2-OH-PhIP-N^3-glucuronide, and 4'-PhIP-sulphate. We have measured the time course of PhIP in untreated and acid- or alkali-hydrolyzed urines from 10 healthy non-smoking subjects ingesting identical amounts of char-broiled beef (containing both HAs and PAHs) for 5 days. The morning after the first day of broiled beef consumption (containing 7.7 μg PhIP), urinary concentration of PhIP increased 14- to 38-fold above mean prefeed concentration. Following cessation of broiled meat consumption, urinary PhIP declined to near prefeed levels within 48–72 h. The ratio of alkali-labile PhIP metabolites to unmetabolized PhIP varied by 2.7-fold among subjects, ranging from 18:1 to 48:1. In a subsequent study we measured PhIP in acid-hydrolyzed urine from 66 subjects ingesting beef pan-fried at high temperature. A significant correlation ($r = 0.61$, $P < 0.0001$) was observed between the amount of fried meat ingested and concentration of PhIP in urines collected between 0 and 12 h after feeding. Other investigators have identified 2-OH-PhIP in acid-hydrolyzed urine from these subjects, and also observed a significant correlation ($r = 0.52$, $P < 0.0001$) with the amount of fried meat ingested. Additional studies have measured PhIP metabolites in subjects consuming their normal (unrestricted) diet. PhIP was detected in acid-hydrolyzed urine from 20 to 50% of these subjects, depending on ethnic group. Taken together, these studies indicate that significant amounts of PhIP are bioavailable from ingestion of fried or char-broiled meats, and that urinary PhIP metabolites reflect

recent (12–24 h) ingestion. Furthermore, significant interindividual differences in the amounts of urinary PhIP metabolite excreted are observed following ingestion of similar amounts of PhIP. These differences do not correlate with interindividual differences in excretion of urinary pyrene metabolites in the same individuals after ingestion of char-broiled beef, indicating that levels of PhIP and pyrene metabolites in human urine are mediated by compound-specific metabolic factors.

1. Introduction

Highly mutagenic heterocyclic aromatic amines (HAs) are formed during broiling or frying of meat and fish due to pyrolysis of amino acids and proteins [1]. More than a dozen HAs have been identified; the most common forms are quinolines, quinoxalines, pyridines, and carbolines [1,2]. In broiled or fried meat, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) is the most common HA, being formed at concentrations of 1–80 μg/kg cooked meat [3,4].

Recent studies have demonstrated the carcinogenic characteristics of PhIP and the enzymes involved in its metabolism. Male rats fed PhIP develop colon adenocarcinomas, whereas female rats develop mammary adenocarcinomas [5]. PhIP is activated to the mutagenic metabolite 2-hydroxyamino-1-methyl-6-phenylimidazo(4,5-b)pyridine (N^2-OH-PhIP) by CYP-1A2, -1A1, and -1B1, or metabolized to an inactive form, 4′-hydroxy-2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (4′-OH-PhIP), by CYP-1A1 and -1B1 [6–8]. These metabolites can subsequently form conjugates with glucuronide, glutathione or sulfate [9,10]. However, in human liver microsomes, glucuronide conjugation with PhIP has also been shown to occur at the N^3 position of PhIP in the absence of hydroxylation [11].

In this review, we summarize investigations of PhIP metabolites in human urine, and describe our studies in which they were monitored, after conversion to parent PhIP, following ingestion of fried or char-broiled beef.

2. Detection and identification of PhIP urinary metabolites

Several HAs, including PhIP, have been detected in the urine of healthy subjects eating normal diets, using ion exchange chromatography and multiple HPLC purification steps [12]. Gas chromatography–mass spectrometry (GC–MS) after chemical derivatization was used to measure urinary HAs in controlled feeding studies of urinary clearance rates [13], the effect of systemic CYP1A2 and *N*-acetyltransferase activities [14,15], or the presence of conjugated and unconjugated HAs [16,17]. Heterocyclic amines have also been measured in human urine by liquid chromatography-mass spectrometry [15,18] or HPLC with fluorescence detection [19] after immunoaffinity chromatography, or by LC–MS–MS [20]. Other studies have administered radiolabeled PhIP and measured radioactivity by scintillation methods [21,22]. Acid or alkali treatment of urine prior to analysis enhances the yield of free PhIP [13,16,17] or 2-OH-PhIP [23] through deconjugation of phase II metabolites. This approach enhances the concentration of detectable compound (PhIP or 2-OH-PhIP) by pooling the major conjugated PhIP urinary metabolites (see later). In addition, one can examine the proportion of hydrolysable PhIP relative to parent PhIP as an index of phase II conjugation. Reistad et al. [16] examined the ratio of hydrolyzable to parent PhIP in urine from eight subjects following ingestion of a fried meat meal and found considerable variation between subjects ranging from 1.4- to 5.8-fold. These results suggested that individual differences in metabolic capacity or induction may influence the relative excretion of PhIP in conjugated and unconjugated forms.

The major metabolites of PhIP detected in human urine are N^2-OH-PhIP-N^2-glucuronide, PhIP-N^2-glucuronide, N^2-OH-PhIP-N^3-glucuronide, and 4′-PhIP-sulphate [20,21] with the first two metabolites accounting for most of PhIP excreted [20]. The relative concentration of these metabolites varies between subjects and may also be influenced by the method of PhIP exposure (pure compound versus fried meat versus normal unrestricted diet).

3. Controlled char-broiled meat feeding study

We have previously shown that the urinary concentration of 1-hydroxypyrene-glucuronide (1-OHP-gluc), a metabolite of pyrene, increased significantly in 10 subjects who had ingested charbroiled ground beef [24]. We subsequently investigated the time course and interindividual variation of PhIP concentration in the urine samples from these 10 subjects. PhIP concentration was determined in both untreated and alkali-hydrolyzed urine to obtain estimates of the proportion of conjugated PhIP metabolites in each subject at multiple time points.

A detailed description of the feeding protocol has been published previously [24]. Informed consent was obtained from ten male non-smoking subjects aged 25–45 who had no known occupational or medicinal exposure to PAHs. During the first 2 weeks of the study, the subjects refrained from eating any CB, smoked, or fried meat. During the third week, 9 oz (252 g) of CB beef was consumed on the first day, and 6 oz (168 g) was consumed on each of the next 4 days. The CB beef was centrally prepared by cooking ground beef patties over charcoal briquets on an outdoor grill until well done. The broiled patties were then homogenized in a blender to a granular consistency and portions weighed (cooked weight) and labeled. The study subjects met daily during the 5 day feeding period and ate the prepared CB beef between 12:00 noon and 2:00 p.m. The subjects again refrained from eating any CB, smoked, or fried meat for 10 days after the CB beef feeding period.

The mean PhIP content of 5 daily CB beef samples was 30.7 ng/g beef (range 25–39 ng/g) corresponding to a daily intake of 7.7 μg on day 1 of feeding and 5.2 μg on days 2–5 (Table 1). First morning voided urine samples (100 mls) were collected before (2 days), during (4 days), and after (4 days) the CB

Table 1
Amounts of charbroiled beef, PhIP, and selected PAHs ingested daily during the feeding study

Day	Beef (g)	PhIP[a] (μg)	Pyrene[a] (μg)	Benzo[a]pyrene[a] (μg)
1	252	7.7	1.7	6.4
2–5	168	5.2	1.1	4.2

[a] Calculated from mean concentrations measured in pooled char-broiled beef samples.

beef feeding period. Urine samples were frozen at $-70\,^{\circ}C$ within 2 h of collection.

An internal standard of PhIP-d_5 was added to each urine and PhIP was extracted with ethyl acetate and purified by solid phase extraction as previously described [17]. For alkaline hydrolysis, samples were incubated in 1N NaOH at $100\,^{\circ}C$ overnight. For analysis by GC–MS, the bis(pentafluorobenzyl)-derivative (BPFB) of PhIP was formed by addition of pentafluoro-benzyl bromide (PFB-Br) in ethyl acetate and diisopropy-lethylamine as described [17]. GC–MS was carried out on a Hewlett-Packard 5980 A gas chromatograph equipped with a splitless injector ($250\,^{\circ}C$) coupled to a Hewlett Packard 5988 A mass spectrometer. The temperature of the GC oven was raised from an initial temperature of 200–$320\,^{\circ}C$ at $30\,^{\circ}C$/min, where it was held for 5 min. Under these conditions, the retention time of the BPFB derivative of PhIP was about 9.5 min. The mass spectrometer was tuned to monitor negative ions at m/z 403.1 [(BPFB-PhIP-d_0)-(PFB)]$^-$ and m/z 408.1 [(BPFB-PhIP-d_5)-(PFB)]$^-$ representing loss of single PFB-groups from the molecular anions. The detection limit for PhIP was 1 pg PhIP/injection and the interassay variability was <16%. Samples with non-detectable PhIP were assigned the value of half the limit of detection, 0.5 pg/ml.

GC–MS analysis of urine samples collected after CB beef ingestion indicated the presence of PhIP at retention time of about 9.5 min in all 10 subjects. This peak co-chromatographed with PhIP standard and demonstrated the expected m/z of 403.1. The baseline concentration of PhIP in unhydrolyzed urine after 2 weeks free of broiled or smoked foods was 0.88 pg/ml urine (range: <1–2 pg/ml, 7 of 20 samples detectable). Mean ($\pm$S.E.M.) urinary PhIP concentration the morning after the first day of feeding was 26.3 ± 6.6 pg/ml urine (Fig. 1) with a range of 5–75 pg/ml. The concentration of PhIP decreased to near baseline levels by 48–72 h after CB beef consumption ended.

The mean ($\pm$S.E.M.) baseline concentration of PhIP in alkali-hydrolyzed urine after 2 weeks free of broiled or smoked foods was 30.4 ± 5.4 pg/ml urine (range 1–84 pg/ml). Mean urinary PhIP concentration the morning after the first day of feeding was 675 ± 97 pg/ml urine with a range of 423–1164 pg/ml. This represented an increase of 14–38-fold above mean baseline concentration ($P = 0.001$ by Wilcoxon rank sum). The 32% decrease in the amount of PhIP ingested on
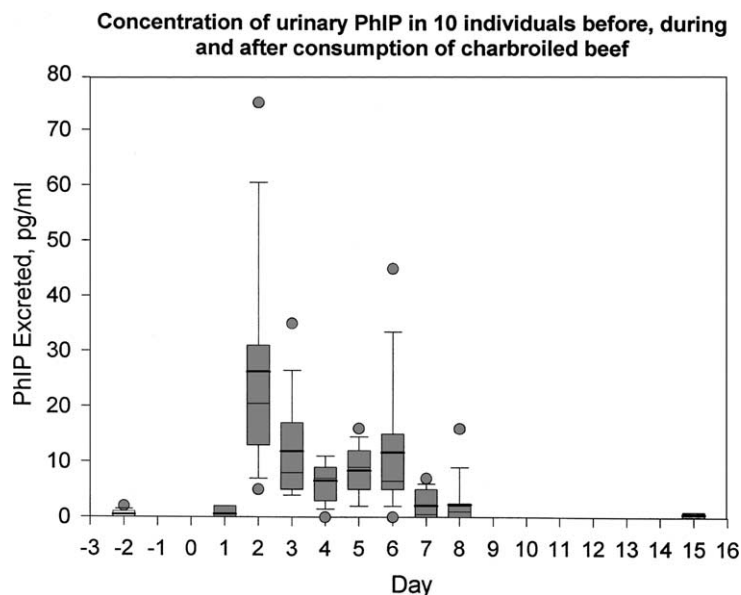
Fig. 1. Concentration of PhIP in unhydrolyzed urine collected before, during and after consumption of CB beef for 5 days. Nine ounces CB beef were consumed on day 1, and 6 oz were consumed on each of days 2–5. Urine samples were collected in the morning before CB beef consumption at midday. Box plots show mean (heavy bar), median, 10, 25, 75, and 90th percentiles of urinary PhIP concentrations on selected days of study.

days 2–5 of the feeding protocol (5.2 μg), compared to day 1 (7.7 μg), was accompanied by a 48% decrease in urinary PhIP concentration on the following mornings. The concentration of PhIP in alkali-hydrolyzed urine decreased to near baseline levels by 48–72 h after CB beef consumption ended. After adjustment for urine creatinine, interindividual differences in mean urinary PhIP concentration during days 3–6 of the study were significant ($P = 0.016$ by Kruskal–Wallis). Overall, creatinine adjustment did not change the rank order or time course of individual urine concentrations of PhIP in hydrolyzed or unhydrolyzed urines.

We next examined, on an individual basis, the correlation of urinary PhIP concentration in hydrolzyed and unhydrolyzed urines collected at 10 time points during the feeding study (Fig. 2). Within each subjects' set of 10 urines, the correlation was quite good (r coefficient ranged from 0.67 to 0.98). The mean ratios of PhIP in hydrolyzed to unhydrolyzed urine (slopes) varied by 2.7-fold among individuals from a minimum of 18:1 in subject E to a maximum of 48:1 in subject A. The urinary PhIP concentrations measured did not

correlate with previously reported levels of 1-OHP-gluc on an individual basis (Fig. 3). That is, subjects with high urinary PhIP levels throughout the study did not necessarily have higher 1-OHP-gluc levels. These findings indicate that interindividual levels of these two metabolites are not mediated by a common factor (e.g. absorption, hydration, physical activity), rather, they appear to be chemical-specific factors. Obvious candidates for these chemical-specific metabolite modulating factors are the phase I and II metabolic enzymes that are involved in the metabolism of PhIP and pyrene. Previous studies have demonstrated the role of CYP 1A1, 1A2, and 1B1 enzymes in the hydroxylation of both PhIP and pyrene. The CYP1 enzymes are inducible and levels of these enzymes are known to vary significantly between individuals. In addition, the phase II enzyme UDP-glucuronosyl-transferase is inducible by a variety of environmental and dietary compounds. Thus, differences in the metabolic capacity of the test subjects could result in different concentrations of metabolites following ingestion of similar quantities of parent compounds. Also, since both HAs and PAHs can induce and be metabolized
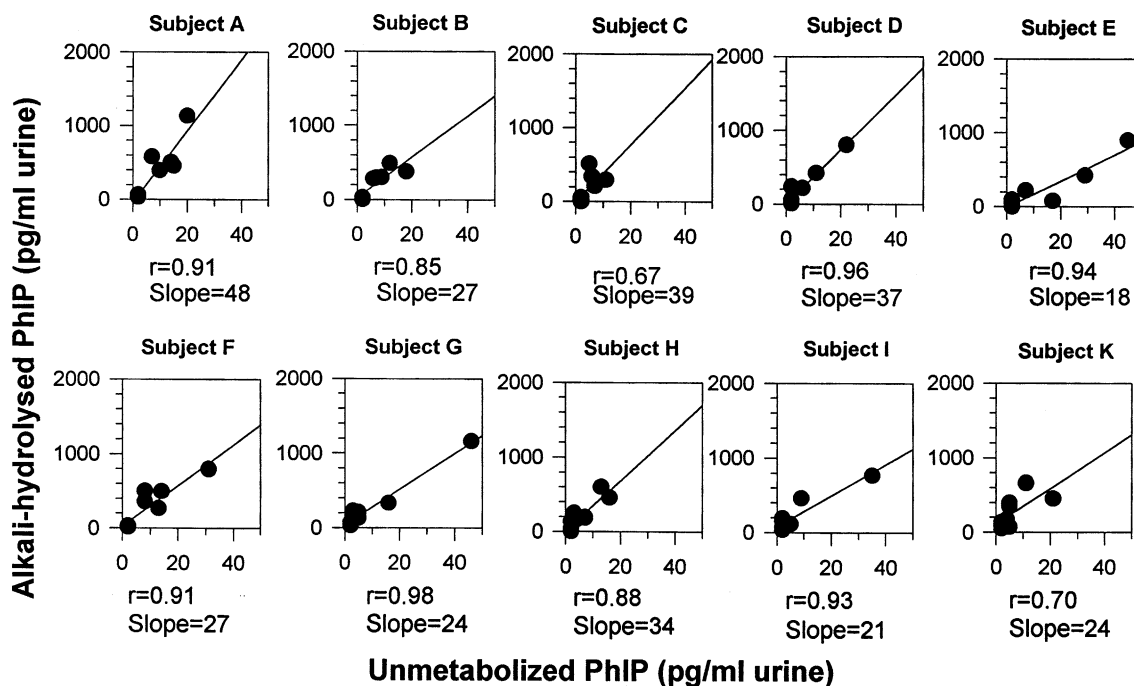
Fig. 2. Individual plots of PhIP concentrations in hydrolyzed urine vs. unhydrolyzed urine for each time point of the 10 study subjects. Individual plots contain 10 time points except plot for subject I which contains nine time points. The slopes are the ratios of free PhIP in hydrolyzed to non-hydrolyzed urine.

by the same enzymes, these compounds may interact to enhance or inhibit their biological effects.

Previous studies of urinary PhIP in humans have measured PhIP either in untreated urine [12,13,25] or hydrolyzed urine [15,18], or both [16]. These measures presumably reflect levels of unmetabolized PhIP, in the former case, and metabolized and/or conjugated PhIP in the latter. We measured PhIP in both alkali-hydrolyzed and unhydrolyzed urine in order to estimate the ratio of conjugated to unconjugated PhIP metabolites. The large increase in the amount of PhIP detected in urine following hydrolysis indicates that labile PhIP metabolites represent a major proportion of the PhIP in human urine. Recent studies of metabolism following ingestion of PhIP indicate that PhIP-N^2-glucuronide, N^2-OH-PhIP-N^2-glucuronide, and N^2-OH-PhIP-N^2-glucuronide are common metabolites in human urine [20,21]. The proportions of these metabolites in our study subjects is under investigation. At the present time we have not definitively identified which metabolite(s) yield PhIP

upon alkali-hydrolysis, however PhIP-N^2-glucuronide is the obvious candidate precursor.

In our study, PhIP was detectable in 35% (7 of 20) of the prefeed hydrolyzed urine samples collected after the subjects had refrained from eating smoked of broiled foods for 2 weeks. This is similar to the percentages reported for subjects on an unrestricted diet [18], and suggests that the method could be amenable to biomonitoring in the general population.

Other controlled feeding studies of meat containing HAs have examined intra- and interindividual variability in internal dose [16,25] and human metabolism by CYP 1A2 [13,14,15] and NAT2 [23]. Reistad et al. [16] determined the ratio of hydrolyzable to parent PhIP in urine from eight subjects ingesting a fried meat meal. They observed a 1.4- to 5.8-fold increase in measurable PhIP following acid hydrolysis of urine in 1N HCl at 100 °C for 2 h. The magnitude of the increases is much lower than those observed in our study. This difference may be due to either the different hydrolysis conditions used (strong acid versus alkali) or the
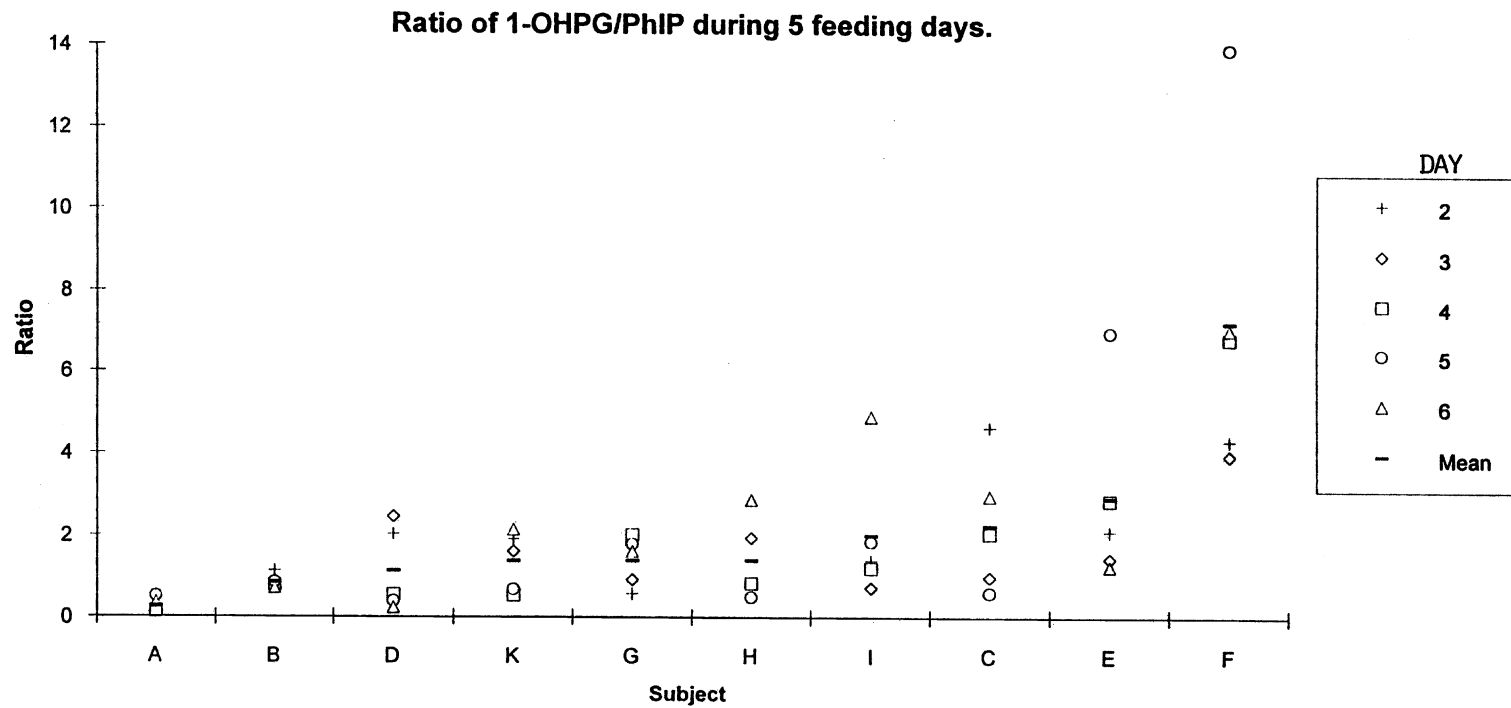
Fig. 3. Ratios of concentration of 1-OHP-gluc to PhIP in acid-hydrolyzed urine from 10 subjects on five mornings (days 2–6) after ingesting charbroiled beef. Mean ratio for each subject is indicated by the solid bar and varied from a maximum of 7.2 ± 1.8 in subject F to a minimum of 0.27 ± 0.07 in subject A.

different cooking methods (frying versus charbroiling) used to prepare the meals. We have found that different hydrolysis conditions greatly affect the yield of free PhIP from human urine samples [19]. We have also reported [24] that the charbroiled meal consumed in our study contained high levels of PAHs in addition to HAs. Since PAHs are known to induce the CYP1A enzymes, they would be expected to alter the phase I metabolism of HAs. As a result, HAs excreted in urine might be expected to contain a higher proportion of metabolized PhIP relative to unmetabolized PhIP.

4. Controlled fried meat feeding study

In this study, we measured PhIP in acid-hydrolyzed urine collected from subjects participating in a study of fried beef ingestion. A detailed description of the fried beef feeding protocol has been published previously [26]. The 66 participating subjects (33 male and 33 female) were healthy non-smokers. The study consisted of two 7-day controlled dietary periods. The first period contained evening meals with meat fried at low temperature (100 °C) producing undetectable levels of HAs, while the second period contained evening meals with meat fried at high temperature (250 °C) producing high levels of HAs. The high temperature beef contained an average of 32.8 ng PhIP/g meat. The amount of beef eaten was adjusted by body weight for each individual and, during the second week of feeding, ranged from 180 to 328 g per day. This corresponded to 5.9–10.8 µg of PhIP consumed per day based on measurement of PhIP concentration in selected meat samples from the study. There were no detectable amounts of PAHs produced in the beef by either of the two frying temperatures. Other details of the controlled diet were described previously [26]. Total urine samples were collected each day in aliquots of 0–12 and 12–24 h after the cooked beef meal. The urine samples used in the current analysis were collected in the 24 h period after the first day of the high temperature diet.

We developed a rapid method to quantitate PhIP in hydrolyzed urine using HPLC with fluorescence detection following immunoaffinity chromatography [19]. Urine samples were hydrolyzed with acid prior to analysis in order to enhance the recovery of PhIP. Thawed urine samples (2 ml) were hydrolyzed by adding 0.5 ml of 0.5N HCl (final concentration 0.1N) and incubating at 90 °C for 60 min. Final concentrations of HCl greater than 0.1N (0.2–1.0N) were also tested. After cooling samples to room temperature, the pH was adjusted to 7.0 with 2N NaOH, and PhIP was purified by solid phase extraction and immunoaffinity chromatography as described [19]. Purified samples were analyzed using an HPLC system with a C18 column and fluorescence detection. Samples were eluted with a linear methanol gradient: 28–60% methanol (over 42 min) in water with 0.1N diethylamine and acetic acid to pH 4 (flow rate 1.0 ml/min). Fluorescence was monitored with a Dynamax FL-1 detector (excitation = 317 nm; emission = 370 nm). Retention time of the major fluorescent peak (28–29 min) was compared with that of a PhIP standard and other PhIP metabolite standards (see [8] for details). The limit of detection of the assay was 0.1 ng PhIP in 2 ml urine (0.22 pmol/ml).

Comparison of the HPLC chromatograms of affinity-purified urine samples collected before and after charbroiled beef ingestion indicated the appearance of a major fluorescence peak with retention time of 28–29 min in all 10 subjects (representative example shown in Fig. 4). This peak co-chromatographed with PhIP standard, demonstrated synchronous fluorescence spectra similar to that of PhIP standard, and was clearly separated from N^2-OH-PhIP and 2-hydroxy-deamino-PhIP standards as shown previously [8]. Representative 28–29 min peaks were collected and analyzed by negative ion chemical ionization GC–MS after chemical derivatization with PFB-Br. This analysis demonstrated the presence of negative ions at m/z 403 confirming the identity of the fluorescent compound as PhIP. In addition, the quantity of PhIP determined by on-line fluorescence was confirmed in a blind comparison with quantity determined in the same sample by GC–MS. The size of the 28–29 min fluorescent peak was 7–10 fold lower when urine samples were not subjected to hot acid treatment. Urine samples treated with final HCl concentrations of 0.3–1.0N showed reduced levels of free PhIP compared to the urines treated with 0.1N HCl indicating that diminished release of PhIP occurred with increasing acid concentration. Thus, optimum conditions for releasing PhIP in urine were found to be incubation at 90° for 60 min at a final HCl concentration of 0.05–0.1N.
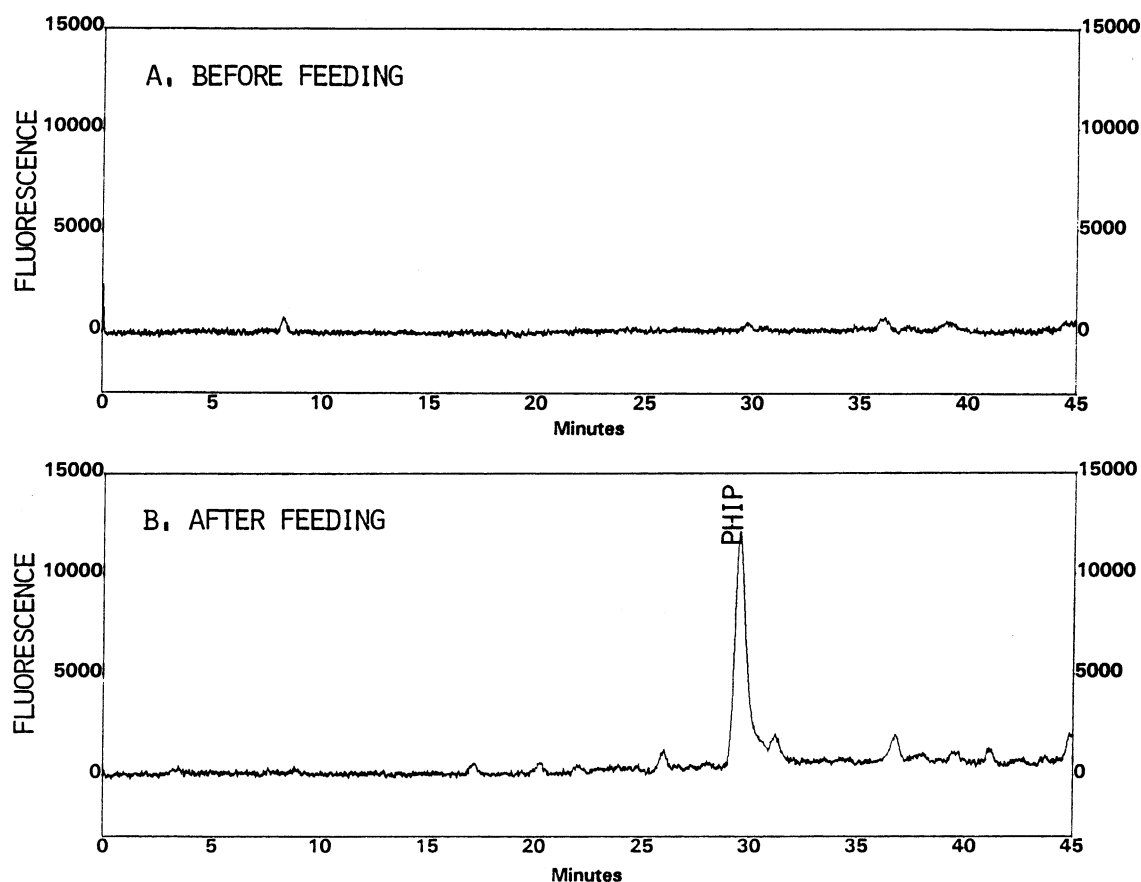
Fig. 4. Representative chromatograms of acid-hydrolyzed urine samples collected before (A) and the morning after (B) CB beef ingestion in one study subject. Samples were purified by immuno-affinity chromatography and analyzed by HPLC with fluorescence detection.

We validated the immunoaffinity chromatography/ HPLC-fluorescence method by comparing results obtained with this method to the previously validated method using gas chromatography/mass spectrometry. The concentration of PhIP in the 100 urine samples from our charbroiled beef feeding study was determined by the HPLC-fluorescence method after acid-hydrolysis of the urine. These results were compared [26] to the concentration of PhIP in the same 100 samples determined by the GC–MS chemical derivatization method after alkaline hydrolysis of urine samples. Because the samples analyzed were collected before, during and up to 4 days after the feeding period, the range of PhIP exposures experienced by the study subjects in the 24 h period prior to each urine sample varied from 0 to 7.7 μg per day. The urinary PhIP concentrations determined by the two methods were highly correlated (Pearson $r = 0.87$, $P < 0.0001$; Spearman $r = 0.75$).

In order to assess the utility of the HPLC-fluorescence assay for detecting urinary PhIP following ingestion of fried meat, we analyzed urines collected from the study of 66 subjects who had ingested different amounts of fried beef cooked at high temperature [26]. Urine samples collected during the time period 0–12 h after the first fried beef meal were analyzed for PhIP after acid hydrolysis. The correlation between urinary PhIP and amount of PhIP ingested by each individual is shown in Fig. 5 and was highly significant (Pearson $r = 0.61$, $P < 0.0001$; Spearman $r = 0.54$, $P = 0.000015$). Similar results were reported by Stillwell et al. [15] showing a significant correlation
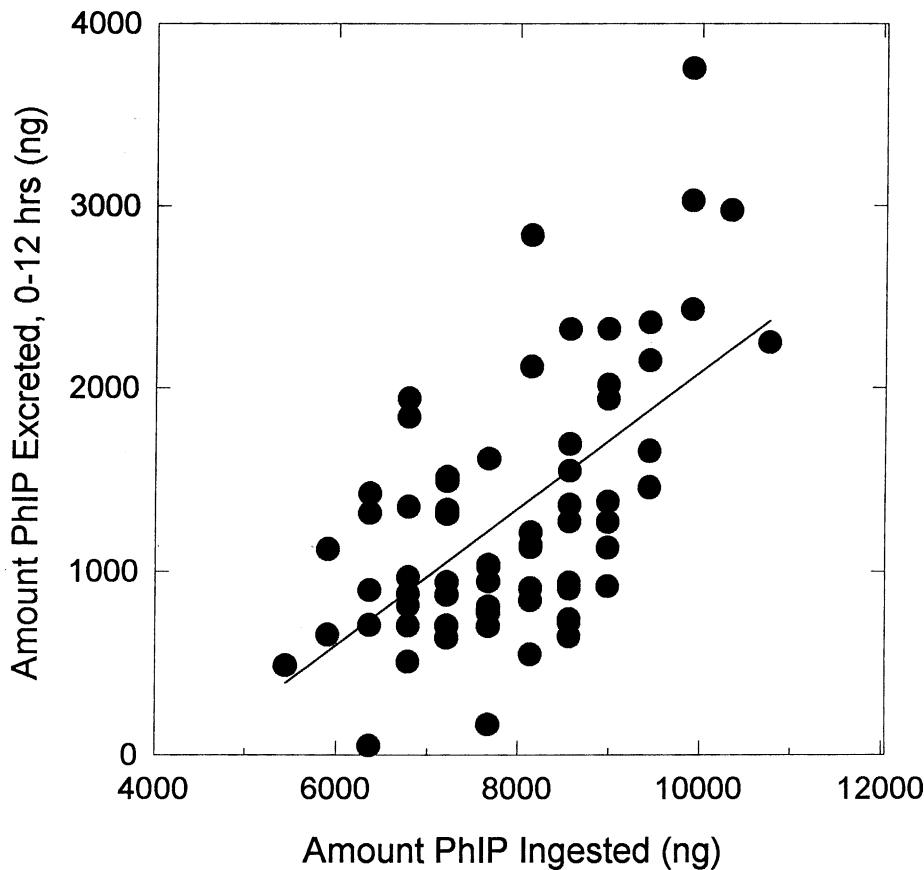
Fig. 5. Concentration of PhIP in 66 urine samples from fried beef feeding study measured by HPLC-fluorescence assay after acid hydrolysis vs. amount of PhIP ingested. Urine collected 0–12 h after ingestion of fried meat.

between the amount of meat consumed and urinary PhIP measured by LC–MS in these samples after acid-hydrolysis under different conditions. They also identified 2-OH-PhIP in acid-hydrolyzed urine from these subjects, and observed a significant correlation ($r = 0.52$, $P < 0.0001$) with the amount of fried meat ingested [23].

We observed no difference between males and females participating in the fried meat study comparing the slope of the best-fit regression lines (not shown), indicating that the dose-response relations were similar. In a subset of six subjects, we also determined urinary PhIP concentration in urines collected immediately before the high temperature fried beef meal and 0–12 and 12–24 h after the meal. PhIP was detectable in 4 of 6 prefeed urine samples analyzed with

a mean ± S.D. of 73 ± 30 pg/ml (range: <50–126 pg/ml urine); this corresponded to a mean urinary PhIP excretion of 116 ± 61 ng in the 12 h prefeed period (range: <35–214 ng). The ratio of PhIP concentrations in the urines collected in the first versus the second 12 h period after ingestion for each subject varied from 3.4:1 to 13.6:1 (mean ± S.D.: 8.4 ± 3.3). Thus, on average, about 89% of PhIP detected in urine was excreted in the first 12 h after ingestion.

The amount of PhIP excreted in the 0–12 h hydrolyzed urine sample represented $16.6 \pm 7.4\%$ of the ingested PhIP. This was three- to four-fold higher than that reported previously [15,16] for controlled feeding studies, and may be due to the different conditions used to hydrolyze PhIP-conjugates. As mentioned above, we found maximal release of PhIP in urine

at final HCl concentrations of 0.05–0.1N, well below those used in the previous two reports (1N). These results indicate that the urine hydrolysis conditions are of critical importance in determining the fraction of PhIP released from putative urinary PhIP-conjugates.

In contrast, Stillwell et al. [23] found that 2-OH-PhIP was the major PhIP-related product in these urines after acid hydrolysis with 0.1N HCl. The amount of 2-OH-PhIP present in the 0–12 h acid-hydrolyzed urine was $20.2 \pm 8.0\%$ of the ingested dose with a range of 5.4–39.6%. Furthermore, they observed an association between concentration of 2-OH-PhIP in urine and CYP1A2 activity ($r = 0.25$, $P = 0.05$ by linear regression) after adjustment for meat intake. This finding is consistent with CYP1A2-mediated N-oxidation of PhIP. These authors propose that 2-OH-PhIP, the deaminated product of N^2-OH-PhIP, is the acid hydrolysis product of N^2-OH-PhIP-N^2-glucuronide.

5. Normal unrestricted diets

Several HAs, including PhIP, have been detected in the urine of healthy subjects eating normal diets [12,18]. Ushiyama et al. [12] detected and quantitated four different carcinogenic HAs in the urine of 10 healthy volunteers eating normal diets, but detected no HAs in the urine of three patients receiving intravenous feeding. Kidd et al. [18] measured PhIP in the acid-hydrolyzed urine from 129 males who consumed an unrestricted normal diet. PhIP was detectable in 51% of African-American subjects, 45% of Asian-American subjects, and only 21% of white subjects. Although the geometric mean levels of urinary PhIP were higher in the Asian-American and African-American subjects than in the white subjects, this difference was not reflected in intake frequencies of cooked meats based on a self-administered dietary questionnaire. A statistically significant association ($P = 0.001$ by χ^2) was observed between PhIP and MeIQx concentrations measured in these samples.

6. Summary

In summary, these studies support the proposal that measurement of PhIP in acid-hydrolyzed urine is use-

ful in assessing recent exposure to this carcinogen. However, the percent of ingested dose excreted as urinary metabolites varies widely between individuals indicating that other factors, such as absorption or metabolic rates, influence the proportion of ingested dose excreted in urine. Another important question in need of further investigation is the relative stability of urinary PhIP metabolites under various hydrolysis conditions. Since different individuals would be expected to produce different relative amounts of these metabolites, variability in PhIP concentration measured in hydrolyzed urine could be due, in part, to differential hydrolysis. Further efforts to refine sensitive methods to quantitate the individual urinary PhIP metabolites are needed, such as the approach developed by Kulp et al. [20].

Acknowledgements

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